

**LONG-TERM USE OF UREA VS. ANHYDROUS AMMONIA  
FOR N-FERTILIZATION OF A DARK BROWN LOAM: III  
SOIL MICROBIAL POPULATIONS AND ACTIVITIES**

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**ABSTRACT**

The influence of fertilizers on soil quality, including soil microbial well-being, has been frequently questioned by proponents of organic farming and LISA. A 10-yr experiment, conducted on a Dark Brown loam at Scott, in which the influence of urea and anhydrous ammonia at rates up to 180 kg N ha<sup>-1</sup> on yields of cereals and oilseeds was examined, provided the basis for this study. In the tenth year we took soil samples at 2 depths (0 to 7.5-, 7.5 to 15-cm) at 3 times (3 days before, 6 days and 26 days after fertilizer application). We assessed the impact of these treatments on populations of filamentous fungi, yeasts, bacteria, actinomycetes, nitrifiers, and denitrifiers. The fertilizer effects were most pronounced 6 days after N application but were also apparent just prior to N application, confirming a residual treatment effect. Generally, the effects were greater in the second depth where the fertilizer was placed. There were no obvious effects on yeasts or denitrifiers. Generally bacterial and fungal populations were directly related to N rates and were increased more by anhydrous ammonia than by urea. In contrast, actinomycete populations were inversely related to rate of N and the population was lower for anhydrous ammonia than for urea. Nitrifiers responded positively to N rate near the depth of N placement with the response peaking at the 90 kg ha<sup>-1</sup> rate. The pH<sub>CaCl2</sub> of the check soil, which was already low (5.2) was further decreased to 4.2 by 180 kg N ha<sup>-1</sup>, the decrease being greater for the anhydrous ammonia source. We believe positive responses in microbial populations are due to the nutritive value of N and negative responses related to soil acidification.

**INTRODUCTION**

The influence of fertilizers, especially anhydrous ammonia, on soil quality, including soil microbial well-being, is being frequently questioned by proponents of organic farming, low input sustainable agriculture (LISA), and some segments of society. Only limited research on the influence of anhydrous ammonia on soil properties has been conducted in Western Canada (Henry et al., 1979). We know of no studies that have examined the influence of anhydrous ammonia on soil microbial activity on the prairies, and only limited research on this subject has been reported elsewhere (Eno and Blue, 1954; Khonje et al., 1989).

Nitrogen fertilizers when used regularly are known to eventually acidify soils (Intrawech et al. 1982; Khonje et al. 1989; Darusman et al. 1991), although urea and anhydrous ammonia will, initially cause an increase in soil pH due to the ammonia released (Eno and Blue 1954). When 100 and 250 lb N/ac was applied as an anhydrous ammonia to a sandy and medium textured soil, the number of fungi was reduced while bacteria and actinomycetes increased except for three days immediately after application when populations decreased in a neutral soil (Eno and Blue 1954). These workers noted however, that from a total population standpoint none of these changes were likely to cause permanent disturbance in the ecological balance of the soil.

The study by Eno and Blue (1954) was short-term. Producers are likely to use the same fertilizer menu on the land, year after year. The question of what would be the long-term impact of the anhydrous ammonia (and other nitrogenous fertilizers) on soil chemical and physical properties has been examined by some

workers (Intrawech et al. 1982; Khonje et al. 1989; Stone et al. 1991; Jacobsen 1992), however, only one such study regarding the influence on soil microbial populations has been reported. In that study, on a silt loam in southern Illinois, Khonje et al. (1989) found no significant changes in the numbers of bacteria, actinomycetes or fungi as the result of nine annual applications of anhydrous ammonia at 150 and at 300 kg N/ha. The check soil in this study had a pH of 4.9 and this dropped to 4.5 when urea was applied and 3.9 when anhydrous ammonia was applied. Even so, this had no apparent negative impact on microbial populations in this low organic matter (1%) soil.

A 10-yr study conducted at Scott, Saskatchewan, (Ukrainetz et al. 1993; Bouman et al. 1993) to measure the response of cereals and oilseeds to several rates of urea and anhydrous ammonia, allowed us to determine the long-term and short-term effects of these treatments on selected soil microbial and biochemical properties.

## MATERIALS AND METHODS

The materials and methods used for this study have been described in this symposium (Ukrainetz et al. 1993; Bouman et al. 1993) therefore only minimum information on this aspect will be provided here. The 10-yr experiment was conducted on an Elstow loam, a Dark Brown Chernozem, to examine the influence of urea and anhydrous ammonia applied to spring wheat and barley in most years and canola in two years, at rates of 45, 90 and 180 kg N ha<sup>-1</sup>. There was also a check (no N) treatments. In the tenth year we took soil samples from 0 to 7.5 and 7.5 to 15 cm depths at 3 days before, and 6 and 26 days after fertilizer application. We measured numbers of aerobic heterotrophic bacteria and actinomycetes by dilution plate count using soil extract agar; filamentous fungi and yeasts were determined by plating on rose bengal-streptomycin agar (Biederbeck et al. 1984); and nitrifiers and denitrifiers were determined by an MPN technique (Biederbeck and Jame, 1982). Determination of microbial biomass, C and N mineralization, and nitrification (Biederbeck et al. 1984) have also been initiated but have not yet been completed. Soil pH was determined in dilute CaCl<sub>2</sub> solution (Bouman et al. 1993).

## RESULTS AND DISCUSSION

### Crop Production and Soil pH

The previous two papers (Bouman et al. 1993; Ukrainetz et al. 1993) have already discussed the influence of these fertilizer treatments on crop production and soil acidity therefore only minimal reference to these factors will be made here.

Wheat yield responses as you recall were positive to 180 kg N ha<sup>-1</sup> for urea, but for anhydrous ammonia responses were lower than for urea and reached their maximum at 90 kg N ha<sup>-1</sup>. The latter depression could be traced to the apparent more efficient recovery of anhydrous ammonia and consequently the lower soil pH that this caused (Figs. 1 and 2). Soil pH was inversely related to rate of N and was lower in the 7.5 to 15 cm depth i.e., the depth of injection (Fig. 1). The impact of fertilizer on pH had occurred several years before we sampled in 1992 and there was little immediate pH response to fertilizer due to the current year's application (Compare values for May 14 and May 5 sampling in (Fig 2).

### Effect on Microbial Populations

The influence of N fertilizer on soil microbial populations can be expected to be tempered by the interaction of fertilizer with nutrient supply (positive impact) and the lowering of pH (negative impact). Microbial responses should be most apparent immediately after fertilizer application and least apparent immediately before fertilizer application. Further, responses should be greater near the depth of fertilizer placement (at least near the time of application), which is in the 7.5 to 15 cm depth. Fungi are known to be able to tolerate soil acidity much better than bacteria, especially nitrifiers (Alexander 1961),

therefore fungi would be expected to reflect the positive influence of nutrients and not be unduly hindered by any acidification caused by the N fertilizers.

We found that microbial populations were, as expected, greater in the surface 7.5 cm than in the 7.5 to 15 cm depth (Table 1). This was particularly true of the bacteria.

Bacterial populations were increased by applied N with the increase being greater at higher N rates only in the 7.5 to 15 cm depth. At the 90 kg ha<sup>-1</sup> N rate bacterial populations were greater for anhydrous ammonia than for urea in the 7.5 to 15 cm depth; this was also true for the 180 kg ha<sup>-1</sup> N rate, 6 days after N application, otherwise there was no effect of N source on bacterial numbers. The results presented for yield (Ukrainetz et al. 1993) and effect on soil chemical properties (Bouman et al. 1993) indicated a more efficient recovery of anhydrous ammonia-N than urea-N but also that this resulted in more acidic conditions under anhydrous ammonia (Figure 2). This would explain the bacterial responses: for example, at the 90 kg ha<sup>-1</sup> N rate the higher bacterial numbers for anhydrous ammonia were in response to the apparent higher N recovery; at 180 kg ha<sup>-1</sup> this positive effect was negated by increased acidity where anhydrous was applied; at 45 kg ha<sup>-1</sup> the amount of nutrients were too low to reflect the differences due to rate or source of N.

At normal rates of fertilizer (90 kg ha<sup>-1</sup> or less) there was no long-term effect of urea on bacterial numbers at the injection depth, but anhydrous ammonia increased bacterial numbers. At above-normal rates of N the positive impact of nutrient level on bacterial numbers persisted throughout the year in the top 15 cm for urea but only at the depth of placement for anhydrous ammonia.

Actinomycete populations, in contrast to bacterial numbers, were generally inversely related to N rate at all times of sampling (Table 1). At the higher N rates, actinomycete populations were significantly lower for anhydrous ammonia than for urea, confirming that actinomycetes are definitely more sensitive to acidity than bacteria (Alexander, 1961). At the 45 kg ha<sup>-1</sup> rate of N there was a temporary positive response by actinomycetes especially at the depth of fertilizer application, likely in response to improved nutrient supply without too deleterious an impact on soil pH.

Fungal response was generally similar to that of bacteria with regards to N rate (Table 1), there being a positive relationship between fungal numbers and N rate. However, the response of filamentous fungi differed from that of bacteria with regards to N source, fungal numbers being greater for anhydrous ammonia than for urea irrespective of time of sampling. Fungal populations were increased soon after fertilizer application, especially at the depth of application, and although in time, populations decreased, the impact of the N fertilizer was still apparent 12 months later. As was to be expected, fungi were able to withstand the acidification caused by N fertilizer quite well and much better than bacteria or actinomycetes and thus these organisms mainly reflected the positive influence of increased N.

Nitrifiers tended to increase with N rate up to 90 kg ha<sup>-1</sup>, and especially when anhydrous ammonia was applied (Table 1). Highest nitrifier numbers were observed at T1 (12 months after application of N). These results reflect a response to conflicting stimuli: the ammonia, as substrate for nitrifiers tends to enhance population growth but higher N rates or more efficient N sources acidify the soil impacting more negatively on populations. As the immediate influence of high concentration of N dissipated, the nitrifier populations were able to reflect the positive influence of substrate enrichment without the negative influence of acidity, particularly at intermediate levels of N. Similarly, Khonje et al. (1989) found no change in nitrifiers at 150 kg NH<sub>4</sub>-N/ha but found a very large reduction in nitrifiers after anhydrous ammonia was applied at 300 kg N/ha.

Yeasts and denitrifier populations showed no obvious effect of the fertilizers (data not shown). Carbon and N mineralization, nitrification and microbial biomass measurements have not yet been completed.

## CONCLUSIONS

These results suggest that anhydrous ammonia is being recovered in the soil system more efficiently than urea and consequently is effectively supplying more N (substrate) to the microorganisms on one hand but also acidifying the soil more than urea on the other. Consequently, frequently there is a positive response of microorganisms to the N substrate up to 90 kg ha<sup>-1</sup> (normal rates of fertilization in this soil) but a negative impact of higher N rates, especially anhydrous ammonia, on most organisms. In contrast to the findings of Eno and Blue, fungi responded well to higher N rates but actinomycetes were quite susceptible. The influence of fertilizer was most apparent near the zone of application but was also apparent in the surface 0 to 7.5 cm depth even 12 months after application.

We conclude that anhydrous ammonia, applied at normal rates, poses no detriment to the soil microflora even when used on a regular basis, but at excessive rates it, and any N fertilizer, will acidify soil and may, therefore pose a serious problem to the ecological balance of the soil's microflora.

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Table 1. Long- and short-term effects of urea and anhydrous ammonia applied at rates up to 180 kg N ha<sup>-1</sup> annually for 10 yr on some microbial populations in surface soil of a Dark Brown Elstow Loam.

Treatments and Significance	---- 0 - 7.5 cm depth ----			--- 7.5 - 15 cm depth ---		
	Sampled on			Sampled on		
	May 5	May 14	June 3	May 5	May 14	June 3
<b>BACTERIA (MILLIONS)</b>						
Check	68	77	90	31	31	24
45 - Ur	77	100	100	24	34	31
45 - AA	65	97	90	36	33	34
90 - Ur	64	112	82	42	33	46
90 - AA	77	99	101	67	65	85
180 - Ur	105	108	108	72	51	70
180 - AA	75	97	110	10	73	78
Prob. Of Significance	0.1	0.39	0.50	0.09	0.001	0.001
LSD (P<0.10)	24	25	26	33	15	12
<b>ACTINOMYCETES (MILLIONS)</b>						
Check	25	15	18	11	9	7
45 - Ur	25	19	17	11	22	8
45 - AA	21	17	14	10	24	8
90 - Ur	24	17	15	11	7	7
90 - AA	21	15	11	6	5	5
180 - Ur	18	19	14	7	4	5
180 - AA	12	11	8	4	2	4
Prob. of Significance	0.02	0.09	0.06	0.001	0.001	0.001
LSD (P<0.10)	6	5	5	3	5	2
<b>FUNGI (Ten Thousand)</b>						
Check	35	36	42	16	31	13
45 - Ur	35	40	49	26	37	24
45 - AA	43	55	58	36	46	32
90 - Ur	45	48	62	29	39	41
90 - AA	51	54	65	42	59	51
180 - Ur	48	57	53	40	49	38
180 - AA	62	78	73	49	63	49
Prob. of Significance	0.01	0.01	0.04	0.01	0.14	0.001
LSD (P<0.01)	12	16	15	13	21	13
<b>NITRIFIERS (Ten Thousand)</b>						
Check	2.1	0.4	0.3	2.4	0.6	0.3
45 - AA	4.5	0.3	1.5	23.4	5.2	27.7
45 - Ur	2.2	0.6	0.7	18.9	2.0	1.6
90 Ur	2.2	1.2	5.7	25.2	15.9	13.2
90 AA	5.0	5.2	9.4	7.0	2.6	5.0
180 Ur	5.1	1.0	2.8	9.6	4.4	30.3
180 AA	0.9	2.0	15.7	3.0	1.1	0.3
Prob. of Significance	0.66	0.28	0.50	0.06	0.02	0.12
	5	4	14	15	6	22

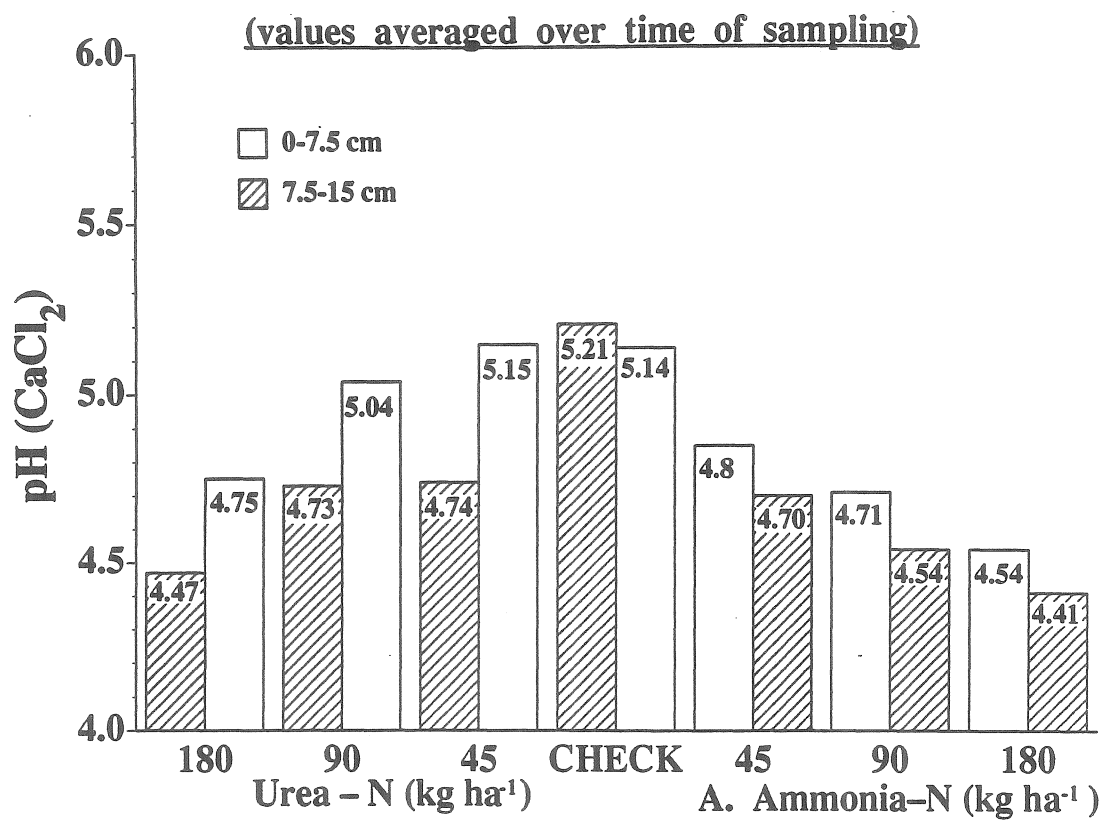


Figure 1. Effect of depth, N source and rate of N on soil pH at Scott (values averaged over time of sampling).

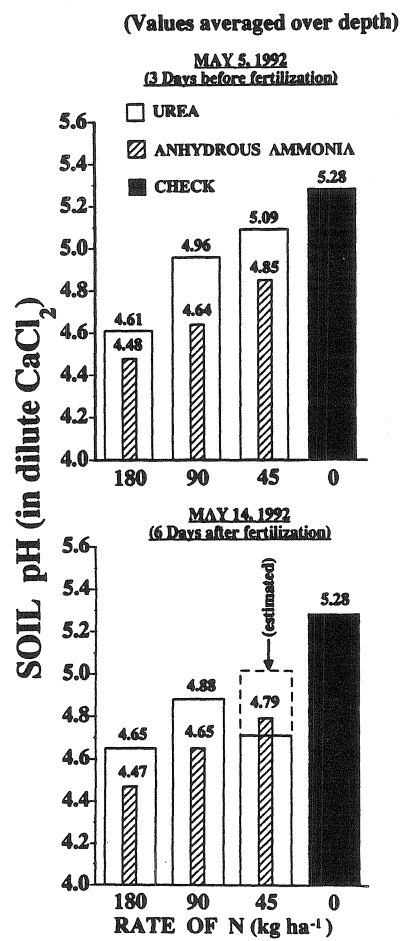


Figure 2. Effect of source and rate of N at different times of sampling (values averaged over depth of sampling).